

SYNNESTVEDT & LECHNER LLP

In re application Darrell Sleep
U.S. National Phase Application
Based on Intl. Application No. PCT/GB2003/003273

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Amendments to the Claims

1. (Original) A polypeptide comprising

- (i) a leader sequence, the leader sequence comprising
 - (a) a secretion pre sequence, and
 - (b) the following motif :

-X₁-X₂-X₃-X₄-X₅-

where X₁ is phenylalanine, tryptophan, or tyrosine, X₂ is isoleucine, leucine, valine, alanine or methionine, X₃ is leucine, valine, alanine or methionine, X₄ is serine or threonine and X₅ is isoleucine, valine, alanine or methionine ; and

- (ii) a desired protein heterologous to the leader sequence.

2. (Original) A polypeptide according to Claim 1 wherein X₁ is phenylalanine.

3. (Currently amended) A polypeptide according to Claim 1 ~~or 2~~ wherein X₂ is isoleucine.

4. (Currently amended) A polypeptide according to Claim 1 ~~any one of the preceding claims~~ wherein X₃ is valine.

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5. (Currently amended) A polypeptide according to Claim 1 any one of the preceding claims wherein the amino acids of the motif are included in the polypeptide as substitutes, for naturally occurring amino acids.

6. (Currently amended) A polypeptide according to Claim 1 any one of the preceding claims wherein X₅ is isoleucine.

7. (Currently amended) A polypeptide according to Claim 1 any one of the preceding claims wherein the motif is -Phe-Ile-Val-Ser-Ile-.

8. (Currently amended) A polypeptide according to Claim 1 any one of the preceding claims wherein the secretion pre sequence is an albumin secretion pre sequence or a variant thereof.

9. (Currently amended) A polypeptide according to Claim 8 wherein X₁, X₂, X₃, X₄ and X₅ are at positions -20, -19, -18, -17 and -16, respectively, in place of the naturally occurring amino acids at those positions, wherein the numbering is such that the -1 residue is the C-terminal amino acid of the native albumin secretion pro sequence and where X₁, X₂, X₃, X₄ and X₅ are amino acids as defined in any one of Claims Claim 1 to 7.

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10. (Currently amended) A polypeptide according to Claim 8 or 9 wherein the albumin secretion pre sequence or variant thereof is a human albumin secretion pre sequence or a variant thereof.

11. (Original) A polypeptide according to Claim 10 comprising the secretion pre sequence MKWVFIVSILFLFSSAYS.

12. (Currently amended) A polypeptide according to Claim 1 any one of the preceding claims wherein the leader sequence comprises a secretion pro sequence.

13. (Currently amended) A polypeptide according to Claim 12 wherein the albumin secretion pre sequence or variant thereof is fused by a peptide bond at its C-terminal end to the N-terminal amino acid of a secretion pro sequence, or variant thereof, thereby to form a pre-pro sequence.

14. (Currently amended) A polypeptide according to Claim 12 or 13 wherein the secretion pro sequence is an albumin secretion pro sequence or variant thereof.

15. (Original) A polypeptide according to Claim 14 wherein the albumin secretion pro sequence is human serum albumin secretion pro sequence or variant thereof.

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16. (Currently amended) A polypeptide according to Claim 14 or 15 wherein the secretion pro sequence motif is the yeast MF α -1 secretion pro sequence or variant thereof.

17. (Original) A polypeptide according to Claim 12 comprising the sequence:

MKWVFIVSILFLFSSAYSRY¹Y²Y³Y⁴Y⁵

wherein Y¹ is Gly or Ser, Y² is Val or Leu, Y³ is Phe or Asp, Y⁴ is Arg or Lys and Y⁵ is Arg or Lys, or variants thereof.

18. (Original) A polypeptide according to Claim 17 wherein Y¹ is Gly, Y² is Val and Y³ is Phe; or Y¹ is Ser, Y² is Leu and Y³ is Asp.

19. (Currently amended) A polypeptide according to Claim 17 or 18 wherein Y⁴ is Arg and Y⁵ is Arg; Y⁴ is Lys and Y⁵ is Arg; Y⁴ is Lys and Y⁵ is Lys; or Y⁴ is Arg and Y⁵ is Lys.

20. (Currently amended) A polypeptide according to Claim 1 ~~any one of claims 1 to 7~~ wherein at least part of said motif is present in the secretion pre-sequence.

21. (Currently amended) A polypeptide according to Claim 1 ~~any one of the preceding~~

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claims wherein the sequence of the desired protein is fused at its N-terminal end to the C-terminal amino acid of the leader sequence.

22. (Currently amended) A polypeptide according to Claim 1 any one of the preceding claims where the desired protein is albumin or a variant, fragment or fusion thereof.

23. (Original) A polypeptide according to Claim 22 wherein the albumin is human albumin.

24. (Currently amended) A polypeptide according to ~~any one of Claims 1 to~~ Claim 21 wherein the mature polypeptide is transferrin or a variant, fragment or fusion thereof.

25. (Original) A polypeptide according to Claim 24 wherein the transferrin is human transferrin.

26. (Currently amended) An isolated polynucleotide comprising a sequence that encodes the motif defined by Claim 1 any preceding claim.

27. (Original) A polynucleotide according to Claim 26 comprising the sequence of SEQ ID No. 15.

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28. (Original) A polynucleotide according to Claim 26 comprising the sequence of SEQ ID No. 16.

29. (Original) A polynucleotide according to Claim 26 comprising the sequence of SEQ ID No. 17.

30. (Original) A polynucleotide according to Claim 26 comprising the sequence of SEQ ID No. 18.

31. (Original) A polynucleotide according to Claim 26 comprising the sequence of SEQ ID No. 34.

32. (Currently amended) A polynucleotide according to Claim 30 or ~~31~~ comprising the sequence of SEQ ID No. 24.

33. (Original) A polynucleotide according to Claim 32 comprising the sequence of SEQ ID No. 25 or a variant thereof, which variant has the leader sequence of SEQ ID No. 24 and encodes a variant or fragment of the albumin encoded by SEQ ID No. 25.

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34. (Currently amended) A polynucleotide according to Claim 30 or 31 comprising the sequence of SEQ ID No. 27.

35. (Original) A polynucleotide according to Claim 34 comprising the sequence of SEQ ID No. 21 or a variant thereof, which variant has the leader sequence of SEQ ID No. 27 and encodes a variant or fragment of the albumin encoded by SEQ ID No. 21.

36. (Original) A polynucleotide comprising the sequence of SEQ ID No. 21 or fragment thereof.

37. (Currently amended) A polynucleotide according to any one of Claim Claims 33, 35 or 36 wherein the polynucleotide comprises a DNA sequence being a contiguous or non-contiguous fusion of a DNA sequence encoding a heterologous protein with either the DNA sequence SEQ ID No. 25 or the DNA sequence SEQ ID No. 21.

38. (Currently amended) A polynucleotide which is the complementary strand of a polynucleotide according to ~~any one of claims~~ Claim 26 to 37.

39. (Currently amended) A polynucleotide according to ~~any one of Claims~~ Claim 26 to 38 comprising an operably linked transcription regulatory region.

40. (Original) A polynucleotide according to Claim 39 wherein the transcription regulatory region comprises a transcription promoter.

41. (Currently amended) A self-replicable polynucleotide sequence comprising a polynucleotide according ~~any one of Claims to Claim 26 to 40.~~

42. (Currently amended) A cell comprising a polynucleotide according to ~~any one of Claims~~ Claim 26 to 41.

43. (Original) A cell according to Claim 42 which is a eukaryotic cell.

44. (Original) A cell according to Claim 43 which is a fungal cell.

45. (Original) A cell according to Claim 44 which is an *Aspergillus* cell

46. (Original) A cell according to Claim 44 which is a yeast cell.

47. (Original) A cell according to Claim 46 which is a *Saccharomyces*, *Kluyveromyces*, *Schizosaccharomyces* or *Pichia* cell.

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48. (Currently amended) A cell culture comprising a cell according to ~~any one of Claims~~
Claim 42 to 47 and culture medium.

49. (Currently amended) A cell culture according to Claim 48 wherein the medium contains a mature desired protein as a result of the production of a polypeptide as defined in ~~any one of Claims~~ Claim 1 to 22.

50. (Currently amended) A process for producing a mature desired protein, comprising (1) culturing a cell according to ~~any one of Claims~~ Claim 42 to 47 in a culture medium wherein the cell, as a result of the production of a polypeptide as defined in ~~any one of Claims~~ Claim 1 to 25, secretes a mature desired protein into the culture medium, and (2) separating the culture medium, containing the secreted mature protein, from the cell.

51. (Currently amended) A process according to Claim 50 additionally comprising the step of separating the mature desired protein from the medium ~~and optionally further purifying the mature desired protein~~.

52. (Currently amended) A process according to Claim 51 additionally comprising the step of formulating the ~~thus separated and/or purified~~ mature desired protein with a

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therapeutically acceptable carrier or diluent thereby to produce a therapeutic product suitable for administration to a human or an animal.

53. (New) A polynucleotide according to any one of Claim ~~Claims 33, 35 or 36~~ wherein the polynucleotide comprises a DNA sequence being a contiguous or non-contiguous fusion of a DNA sequence encoding a heterologous protein with either the DNA sequence SEQ ID No. 25 or the DNA sequence SEQ ID No. 21.

54. (New) A polynucleotide according to any one of Claim ~~Claims 33, 35 or 36~~ wherein the polynucleotide comprises a DNA sequence being a contiguous or non-contiguous fusion of a DNA sequence encoding a heterologous protein with either the DNA sequence SEQ ID No. 25 or the DNA sequence SEQ ID No. 21.

55. (New) A process according to Claim 51 additionally comprising the step of further purifying the mature desired protein.

56. (New) A process according to Claim 55 additionally comprising the step of formulating the thus separated and purified mature desired protein with a therapeutically acceptable carrier or diluent thereby to produce a therapeutic product suitable for administration to a human or an animal.